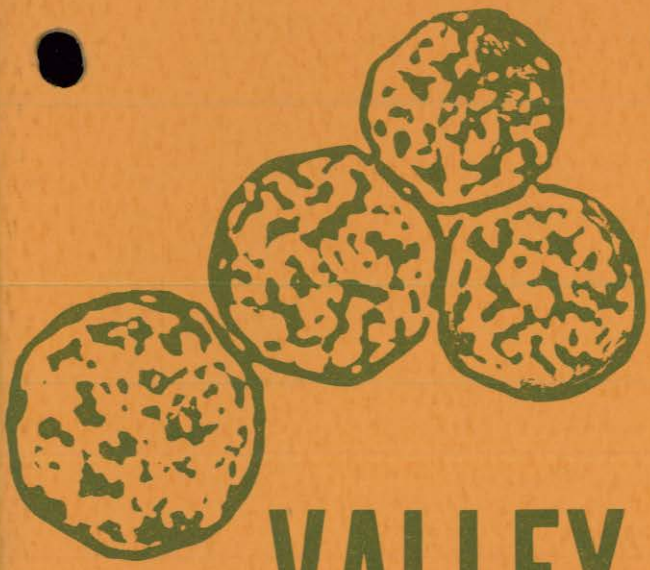
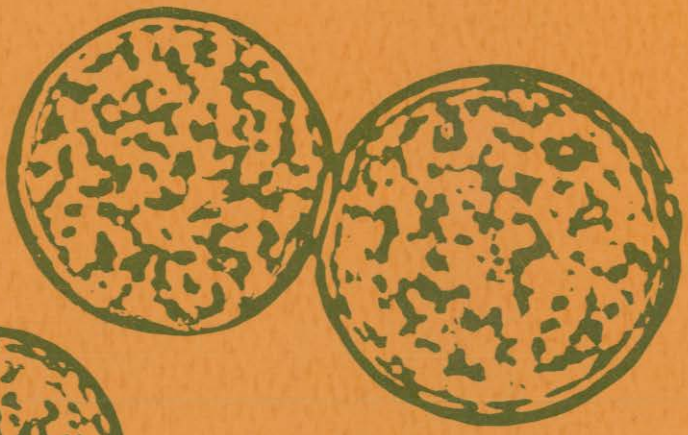


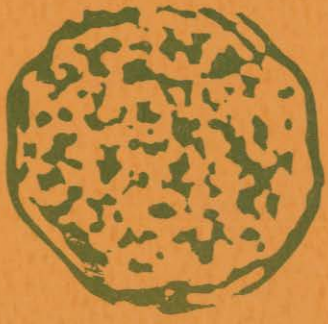
OFFICE OF
NAVAL RESEARCH

BUREAU OF
MEDICINE AND SURGERY

VALLEY FEVER

Studies conducted at the
University of California
Naval Biological Laboratory
Oakland, California 94625





A REVIEW OF NAVY SPONSORED
STUDIES ON AN EXPERIMENTAL
VACCINE FOR COCCIDIOIDOMYCOSIS

Virtually every scientific investigation draws upon the findings of earlier workers. I was additionally fortunate in having guidance and advice from highly competent scientists associated with the Naval Biological Laboratory and other institutions. I express my thanks to many who have encouraged me and helped me in this study. In particular I'm grateful to three colleagues whose contributions, direct or indirect, appear without acknowledgment on every page: the late Doctor C.E. Smith, Doctor Demosthenes Pappagianis and Doctor Yi-chi Kong. And to my wife, Rose, go my warmest feelings for sharing every phase of this ten-year study with me.

Hillel Levine
Chief, Mycology Division

Naval Biological Laboratory
June 27, 1968

THE PROBLEM

The southwestern region of the United States has a problem that is occasionally felt throughout the country and, indeed, the world. The problem is caused by a fungus that grows in the soil covering wide areas of California, Arizona, New Mexico and Texas. The fungus is pathogenic, producing serious disease in man. And because it occurs over thousands of square miles of land, it can't be eradicated. Distant regions and countries often first learn about the problem when illness arises in their residents who have visited our endemic zones, or when fungus-contaminated products exported from the Southwest infect distant recipients.

Strangely, however, the fungus, called *Coccidioides immitis,* does not colonize new territories and so its major public health significance is restricted, in the United States, to the Southwest. There are endemic foci, also, in Mexico and Central and South America. The general name for the disease produced by *Coccidioides* is coccidioidomycosis or, more popularly, Valley Fever, because of its frequent occurrence in the San Joaquin Valley of California. Despite the name of the illness, its symptoms and consequences may involve much more than fever.

LOWER SONORAN LIFE ZONE OF THE UNITED STATES (approximate).

1. The known areas of coccidioidal endemicity occur within the boundaries of this zone:
   - A. Arid to semi-arid climate
   - B. Alkaline soil
   - C. Relative freedom from severe frosts
   - D. Prevalence of the creosote bush *(Larrea tridentata)*
2. Highly endemic coccidioidal areas correlated with locales that are:
   - A. Hot and dry for several months(120% to 32% C, mean summer temperture) (26°-32°C)
   - B. Winter wet season of 12.7 to 31 cm annual mean rainfall (Bakersfield, Fresno, Phoenix, El Paso)

VARIETIES OF VALLEY FEVER AND POPULATIONS AT RISK

In its most gentle form, Valley Fever is symptomless, and at its most savage, it kills and maims. An estimated 85,000 Americans yearly are exposed to the fungus of Valley Fever; the immediate cause is almost invariably the same--breathing contaminated air. During the warm, dry months of the year, Valley Fever spores are discharged into the air and reported illnesses mount in May and June and continue through September to November. To be sure, there are sporadic cases of infection contracted from soil-contaminated wounds and scratches, but the primacy of the respiratory route of infection is clear.

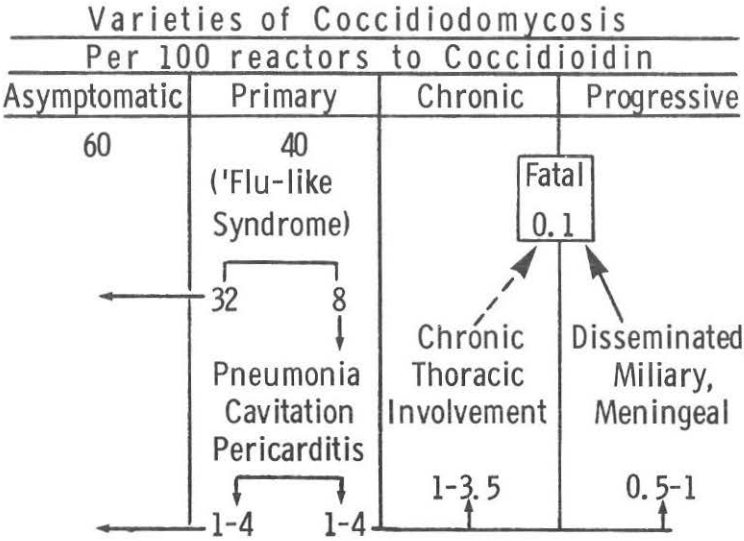
All segments of the population in regions where the fungus is prevalent are at risk, but those in agricultural pursuits, who have close contact with the soil, and people undergoing military field-training, often show a particularly high incidence of disease. Medical officers have reported that at Williams Air Force Base, near Phoenix, the cost of Valley Fever, in terms of man-days of hospitalization, greatly exceeded the combined corresponding costs of the three most frequent acute illnesses--tonsillitis, upper respiratory infections and gastroenteritis--plus all traumatic injuries!

Fortunately, most coccodioidal infections are not severe; approximately 60% of infections pass unnoticed or are accompanied by transitory symptoms of malaise. In these cases, the infection is usually diagnosed retrospectively by a coccidioidin skin test. However, approximately 40% of Valley Fever infections produce moderate to severe pulmonary disease, in which ¼ of the patients require medical attention and, in approximately 3% of all coccidioidal experiences, prolonged chronic sequellae, sometimes leading to death, ensue. The striking public health importance of these percentages becomes clear when it is appreciated that vast populations are exposed; between 80 and 90% of residents in the areas of Bakersfield, Phoenix and El Paso show skin reactivity to coccidioidin which is indicative of exposure.

It is not the purpose of this description to outline the pathologic manifestations of advancing Valley Fever. Suffice it to say that virtually every tissue of the body, including the nervous system, may become involved and be damaged irreversibly. The only treatment is administration of an antibiotic, Amphotericin-B, which is usually restricted in use to advanced or potentially lethal cases because the antibiotic is itself toxic and may produce very serious, permanent kidney malfunction.

THE RATIONALE FOR A VACCINE SEARCH

Skin sensitivity to coccidioidin, indicative of contact with *Coccidioides,* was studied very extensively by the late Dr. C. E. Smith of the School of Public Health, University of California, Berkeley. After determining the reaction of literally thousands of inhabitants in California's San Joaquin Valley and recording their medical history, an exciting relationship became evident: Once a patient recovered from Valley Fever,

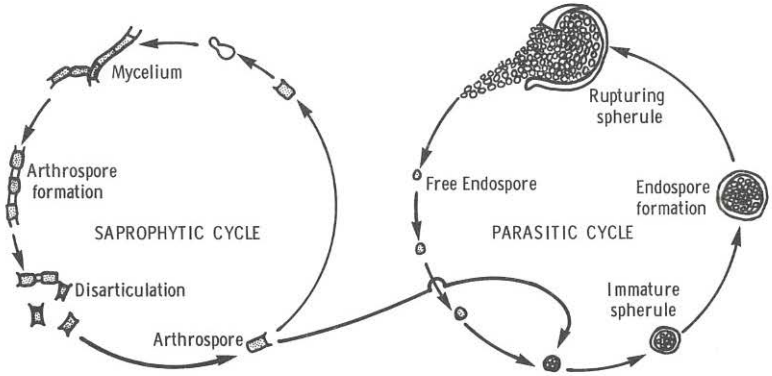


or even if an infection was totally asymptomatic, there was virtually no subsequent history of symptomatic coccidioidomycosis. And this was so, despite the likelihood that exposure occurred time and time again. The excitement of the observation lay in the inference, unrecognized before Dr. Smith's study, that man was capable of an effective immunologic response to *Coccidioides* and hence successful vaccination was at least potentially feasible.

A WORD ABOUT MYCOLOGY

Two characteristics of *Coccidioides immitis* should be described to clarify the approach used in a search for a vaccine. The first is that the organism changes its shape and form when it leaves the soil (saprophytic existence) and invades living tissue (parasitic existence). The second is that the different forms have different properties.

In the soil, or in lifeless laboratory nutrients, *Coccidioides* grows in a threadlike form called a "hypha" to form a mat or "mycelium". Within the threads, tough structures called "arthrospores" are formed. When dry, the threadlike structure fractures and the arthrospores are released. They readily become airborne. If they land elsewhere in the soil, they generate new hyphae and the cycle is repeated.



DIFFERENT FORMS OF COCCIDIOIDES IMMITIS

But if the arthrospores are inhaled and lodge in the lung, they become round and enlarge to many times their original volume. They are now called "spherules" and within the body of the spherules, small living structures termed "endospores" develop. In time the spherules rupture and the endospores are released; they become blood-borne and lymph-borne, and are carried to other loci in the body to generate new spherules. In this manner, *Coccidioides* may colonize healthy tissues to produce generalized disease.

THE SPHERULE VACCINE

A perplexing feature was that early vaccines afforded little protection. Scientists of the U. S. Naval Biological Laboratory found killed mycelial or arthrospore preparations did not protect animals well when they were subsequently infected or challenged with living arthrospores. This was so despite the observations that Dr. Smith made of human beings and despite similar experimental findings that animals surviving infection became strongly immune.

It was only after years of research work that the reason for the inadequacy of the vaccines became apparent: The killed mycelial and arthrospore structures were poorly endowed with protection-inducing antigens or "immunogens". Accordingly, the spherule was investigated for its immunologic efficacy. Naval Biological Laboratory scientists modified a spherule growth medium developed by Army scientists and used it with a virulent strain of *Coccidioides* designated "Silveira". Methods and procedures were then devised to free the spherules of mycelial and arthrospore structures so that valid comparisons of the immunizing properties of each could be made.

The initial comparisons were made in three groups of mice vaccinated with killed mycelia, arthrospores or spherules and later challenged with 127 to 185 arthrospores. A fourth group of challenged mice had received no vaccine at all. The mortality results after a 3- to 6-month observation period were striking:

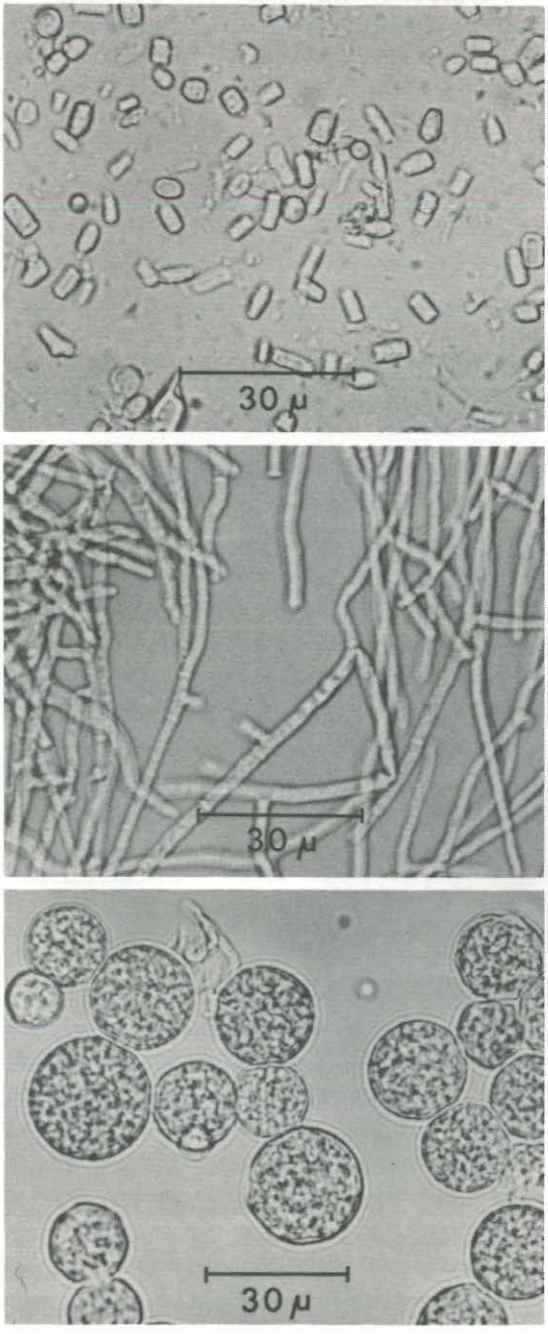
Per cent death (19-23 mice/group)			
Nonvaccinated	Spherule-vaccinated	Mycelium-vaccinated	Arthrospore vaccinated
83 %	11 %	50 %	57 %

Over a period of three years, the spherule vaccine was improved such that now virtually all spherule-vaccinated mice survive challenge doses in the magnitude of 800 to 1000 arthrospores, whereas 90% of nonvaccinated animals succumb to a challenge dose of 70 to 100 arthrospores.

Recently, in a joint study conducted by the Naval Biological Laboratory and the Veterans Administration Hospital in San Fernando, California, mice vaccinated with killed strain Silveira spherules were challenged with aberrant strains of *Coccidioides.* Five such strains, almost unrecognizable as *Coccidioides,* as well as two typical strains, were used in the study. The mice were well protected in all cases, suggesting that strain differences are not likely to be too important a feature in a mass vaccination trial.

SCOPE OF THE MONKEY TRIAL

After the vaccine had been tested in many hundreds of mice, a trial was conducted in *Macaca irus* (Cynomolgous) monkeys to determine if vaccination also protected primates from death after challenge. Ten nonvaccinated and nine spherule-vaccinated animals were exposed to aerosolized *Coccidioides immitis* arthrospores under conditions which were calculated to permit each to inhale approximately 200 arthrospores. Within 9 months, 7 of the nonvaccinated were dead or moribund. Only one of the vaccinated animals died; a second was inadvertently killed during removal of blood by cardiac puncture. X-ray analysis as shown below suggested the mechanism of protection. Disease, evidenced by widespread shadows on the lungs, was prevalent in the nonvaccinated groups (C series). In contrast, few such detectable lesions were seen in the chests of the vaccinated monkeys (V series). Apparently, immunization enabled the animals to contain the infecting organisms in small lesions and somehow prevented the disease from spreading.



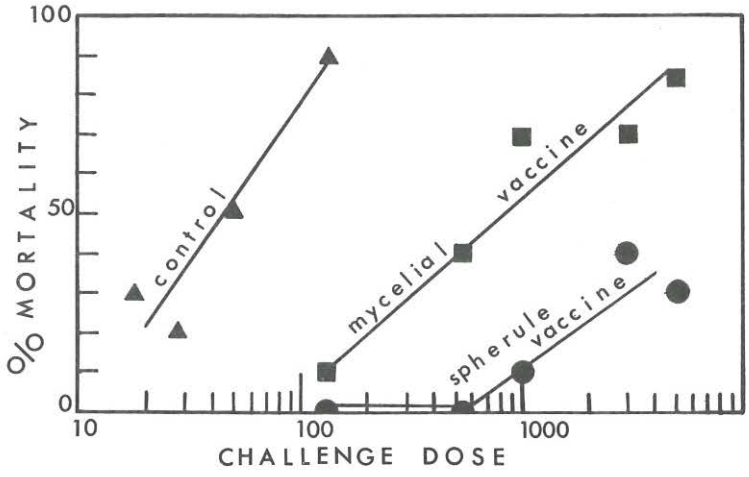
Anthrospores (top), Mycelia (center) and spherules (bottom) of *Coccidioides immitis.*

CONTAINMENT OF INFECTION

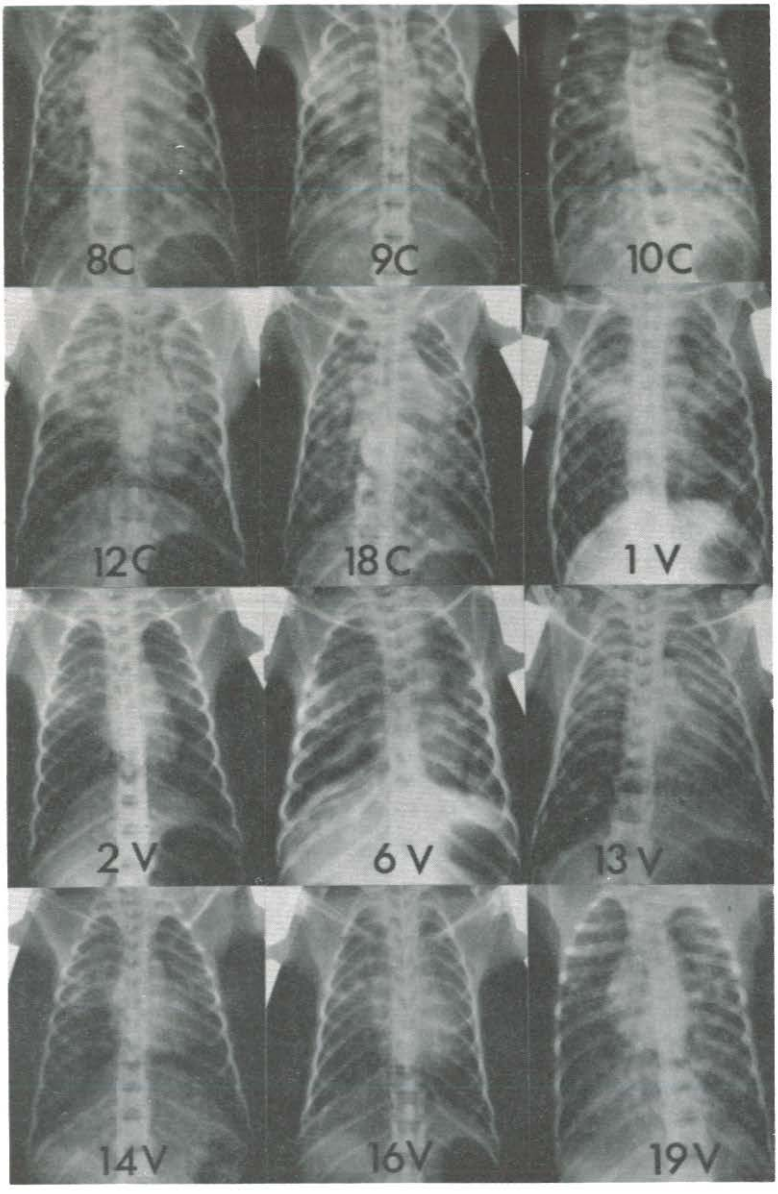
In both mice and monkeys, vaccination prior to challenge produced three profound effects: (1) Life was preserved; (2) disseminated infections (involvement of tissues or organs other than the lung) were either prevented (mice) or minimized (monkeys), and (3) the extent of fungal growth was limited.

Microscopic studies also showed that vaccination predisposed the animals better to contain *Coccidioides* in well-organized lesions. Typically, the lesions of vaccinated mice and monkeys were fewer and smaller than in the nonvaccinated and fewer of these lesions were disintegrating, or undergoing necrosis. Additionally, the lesions of the vaccinated animals contained fewer organisms and had superior architectural organization (round cell infiltrate and fibrosis) than those of nonvaccinated animals. And, finally, the process by which the lesion became architecturally organized to contain *Coccidioides* occurred more rapidly in the vaccinated than in nonvaccinated animals.

Vaccination did not prevent infection; it minimized the consequences of infection. The animals were then able, over a period of many months, to eliminate or markedly reduce the number of *Coccidioides* cells in their tissues.



Mortality in vaccinated and nonvaccinated mice challenged with *Coccidioides immitis*



X-Ray plates of vaccinated (V) and nonvaccinated (C)
Monkeys made at 207 days after infection.

WHERE ARE THE IMMUNOGENS?

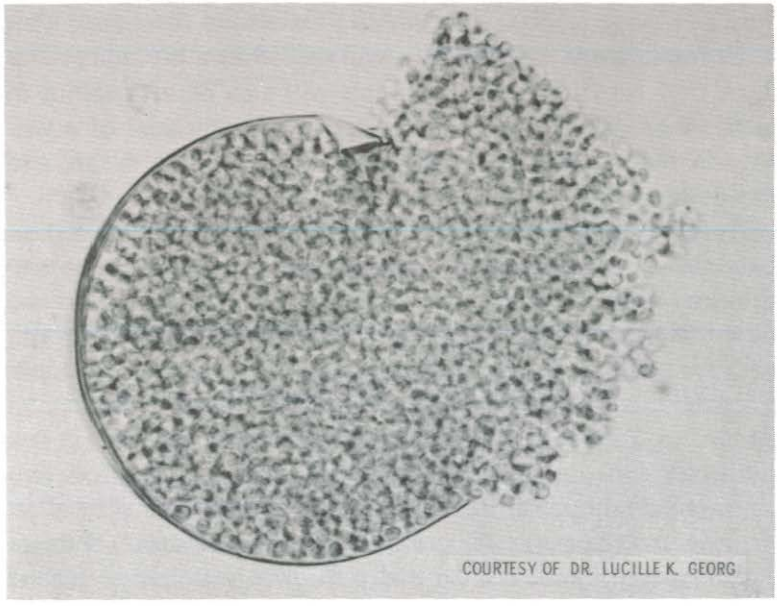
The spherule structure is large (up to 80 microns in diameter) and organizationally complex. It contains many hundreds of endospores within a doubly refractile, thick-walled housing.

A project was undertaken at the Naval Biological Laboratory to determine the location and chemical nature of the immunity-inducing substances, or immunogens. An answer was obtained to the first part of the question, but not to the second part.

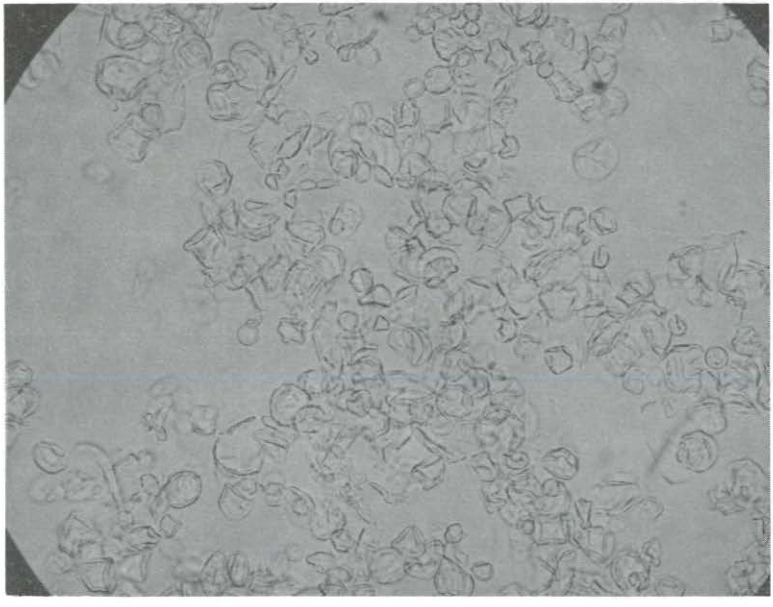
It was found that when the spherules were ruptured, three cell fractions could be separated for study. These were the soluble components, the endospores, and a particulate fraction, presumed to be fragments of the wall. The soluble fraction and the endospore fraction were totally inactive as vaccines. However, the particulate fraction induced strong immunity.

Procedures were then devised to remove the wall of the spherules in such a manner that the walls were essentially intact and identifiable as walls when viewed through the microscope. The walls were washed and freed rather completely of other cellular structures. Used as a vaccine, these induced strong immunity. Moreover, when the walls were mixed with the rest of the spherule components, the immunity induced by the mixture was no stronger than that induced by the isolated walls themselves. For this reason we concluded that the wall of the spherule was the major residence of the immunogens.

As yet we've failed to characterize the immunogens chemically. To accomplish this it is necessary to isolate them, in soluble form, from the wall. All procedures thus far employed--chemical, physical and enzymological--have not yielded a satisfactory soluble preparation. But several properties of the immunogens were determined during these studies. There is a heat labile immunogen and one that is very stable to heat. Chemical and immunological analyses suggest that the former is a protein and the latter a complex sugar; optimal results occur only when both are represented in the vaccine.



Rupturing spherule of *Coccidioides immitis;* impression smear from an infected guinea pig.



Purified walls of *Coccidioides* spherules.

FIRST HUMAN STUDIES

Unfortunately, there is no assurance that a biological product that is efficacious in animals will also be efficacious in man. A necessary step that must precede any trial of a vaccine in man is that of determining if the vaccine is safe and the dosage that may be injected safely.

In November, 1961, scientists of the U. S. Naval Biological Laboratory volunteered as "guinea pigs" for testing the vaccine and eight were selected. At first very small doses of the spherule vaccine, 0.2 mg, were injected into the volunteers who were then observed for a month. There was no ill effect. Gradually the dosage was increased, by very small increments, to 2.7 mg. Still there was no ill effect although one volunteer developed transient hives which lasted for two days. It was later learned that this individual was hyper-allergic also to other stimuli: grasses, strawberries, beer, changes in temperature and that he had a flu-like response on receiving another vaccine several years earlier. He reported further, that his one brother has similar allergic responses.

Observing that the vaccine was safe in dosages up to 2.7 mg, 59 paid volunteers were obtained at a prison facility to extend the study. Proceeding very cautiously, over a four-year period, an answer was found to the question of safe dosage. At doses up to 5 mg the vaccine was well tolerated although there was occasional mild tenderness and slight swelling at the inoculation site. At doses in excess of 5 mg there was local discomfort and swelling which lasted for 7 to 10 days. Some who were sensitive to the fungus because of a prior infection (i.e., coccidioidin-positive skin test) developed a transitory mild fever. This indicated that dermal reaction should be ascertained prior to vaccination.

The study thus provided an estimate of the dosage that could be tolerated comfortably by man and it also showed that the vaccine was safe. However, no data on the vaccine's possible efficacy in man was obtained; the study was not designed for that purpose. And, indeed, information on the usefulness of the vaccine for man can only be gleaned from a

very long-term study because of two intrinsic problems pertaining to human Valley Fever. These are discussed in the next section.

FOR THE FUTURE

Ideally, in a human trial to determine the utility of a vaccine, two large groups of volunteers would be required. Cooperating physicians would treat the volunteers either with the vaccine or an innocuous preparation termed "placebo". The containers for both would be coded such that neither the physician nor the volunteer would be aware of the contents. At a later date all volunteers would be infected deliberately and the course of the disease would be followed.

This ideal procedure cannot be considered in the case of Valley Fever nor is the above idealization intended to recommend it, because there is no safe and sure therapy to terminate the illness.

An alternative approach would be to treat, as above, groups of people who reside in endemic regions and to follow their subsequent clinical history. It would be important to select only those who have had no prior contact with *Coccidioides.* Inevitably, some would become infected by virtue of their residence in the endemic zones. The extent and ratios of clinically manifest illness in both groups would then provide an index of the vaccine's usefulness for man. Conceivably, personnel from outside the areas of endemicity arriving for military training at bases in the region could be utilized for such a trial.

Still, two intrinsic problems, alluded to earlier, remain. The first is that only 40% of the nonvaccinated volunteers can be expected to contract illness and in only approximately 25% of these will the illness be sufficiently severe to require medical attention. Thus, for the findings to have statistical meaning, the initial numbers of volunteers would have to be very large. The second problem is that the time of exposure can't be predicted; it may occur shortly after arrival of the volunteer in an endemic region or only after many years. Hence the clinical followup would have to be long-term.

Since both military and civilian populations in this country are mobile, difficulties in followup can be anticipated. Nevertheless, despite these problems, the study is feasible and, in the judgment of Naval Biological Laboratory scientists, worthwhile. It is to be considered for the future.

GENERAL

A by-product of this study on a vaccine for Valley Fever has been the development of a model experimental system to study antifungal immunology. Currently, there are no other fungal diseases for which an effective experimental vaccine has been developed. It is hoped that the information and techniques learned through this research will help to open other doors in fungal prophylaxis. An immediate tangible result of the work is that a tool is provided by which the immune mechanisms that operate in experimental coccidioidomycosis of animals can be investigated.

ACKNOWLEDGMENTS

The funds for the experimental investigations outlined in this report were provided by the Bureau of Medicine and Surgery and the Office of Naval Research, United States Navy. The Armed Forces Experimental Epidemiology Board supplied funds for the studies in prisoner volunteers. The Naval Biological Laboratory gratefully acknowledges the support of these agencies and expresses its gratitude to the American Public Health Association for honoring the study with an award. The gracious interest of many universities and laboratories, here and abroad, which have invited Naval Biological Laboratory scientists to lecture on the findings is also mentioned with thanks.

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